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Cortinarius bovarius (Agaricales), a new species from western North America

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Abstract

Cortinarius bovarius **sp. nov.**, a conifer associated taxon growing on calcareous ground, is described from western North America. Phylogenetic relationships and species limits were investigated using rDNA ITS and nuclear *rpb2* sequences, morphological and ecological data. The species belongs to section *Bovini* and its closest relative is European *C. bovinus*.

Key words

ITS, MrBayes, *rpb2*, taxonomy, *Telamonia*

Introduction

Cortinarius is the most species rich genus of the Agaricales with a worldwide distribution. *Cortinarius* species are important ectomycorrhizal fungi associated with different trees and shrubs belonging e.g. to the order Fagales and families Pinaceae and Salicaceae. Lately it has also been suggested that they have a key role in the carbon cycling of boreal forests (Bödeker et al. 2011).

In recent years there have been a number of publications on the taxonomy, evolution and biogeography of species found in North America (Seidl 2000, Moser and Peintner 2002, Matheny and Ammirati 2006, Garnica et al. 2009, 2011, Bojantchev 2011a, b, Bojantchev and Davis 2011, Harrower et al. 2011, Ammirati et al. 2012, 2013, Niskanen et al. 2012, 2013a, in press a). These studies show several patterns of species distributions. There are species common to North America and Europe, especially those species from more northern and montane conifer forests, i.e. *Corti-*

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narius aureofulvus M. M. Moser and *C. napus* Fr. There are also presumably endemic species in both Western North America, eastern North America and Europe, i.e. *C. elegantio-occidentalis* Garnica & Ammirati and *C. californicus* A.H. Sm. in western North America, *C. hesleri* Ammirati, Niskanen, Liimat. & Matheny in eastern North America, and *C. puniceus* P.D. Orton in Europe.

Niskanen et al. (in press b) studied *Cortinarius bovinus* Fr. and morphologically similar species occurring in boreal coniferous forests in rich forest soils in northern Europe. Seven species were recognized, all belonging to section *Bovini* (subgenus *Telamonia*). Four of them, *C. bovinus* Fr., *C. bovinaster* Niskanen, Kytöv. & Liimat., *C. bovinatus* Kytöv., Liimat., Niskanen & H. Lindstr., and *C. oulankaënsis* Kytöv., Niskanen, Liimat. & H. Lindstr., formed a well-supported (PP 1.00) clade inside sect. *Bovini* (*Bovini* s. str.). The species are characterized by brown to dark brown basidiomes without bluish colors and exsiccatae with a dark brown to blackish brown pileus. The universal veil is white, brownish white or grayish white, in some species becoming grayish brown with age, and the odor is indistinct or slightly raphanoid. To date, species are only known from Europe, except *C. oulankaënsis* which also occurs in Canada in British Columbia. By studying more material from western North America, we wanted to determine if *C. bovinus* found from Alaska, U.S.A. and Alberta, Canada is conspecific with European samples or does it represent an autonomous species.

Methods

Material gathered by the authors from North America was studied morphologically, ecologically and sequenced to infer phylogentic relationships with other species in *Bovini*. DNA was extracted from dried material (a piece of lamella) with the NucleoSpin Plant kit (Macherey-Nagel, Düren, Germany). Primers ITS 1F and ITS 4 (White et al. 1990, Gardes and Bruns 1993) were used to amplify ITS regions, and specific primers cort6F and b7.1R (Frøslev et al. 2005) for the *rpb2* region. The same primer pairs were used in direct sequencing. PCR amplification and sequencing followed Niskanen et al. (2009). Sequences were assembled and edited with Sequencher 4.1 (Gene Codes, Ann Arbor, Mich., USA). Using a BLAST query of the public databases (GenBank: http://www.ncbi.nlm.nih.gov/ and UNITE: http://unite.ut.ee/), we checked if identical or similar sequences for our species exist in public databases. For the phylogenetic analysis ITS and *rpb2* sequences of the species belonging to the well-supported ingroup of section *Bovini*, *C. bovinus, C. bovinaster, C. bovinatus*, and *C. oulankaënsis*, were included. *Cortinarius anisatus*, *C. neofurvolaesus*, and *C. sordidemaculatus* were chosen as outgroup species.

The combined ITS and *rpb2* alignment of 11 specimens was produced with the program MUSCLE (Edgar 2004) under default settings. The alignment comprised 1286 nucleotides (including gaps). The alignment is available at TreeBASE under S14159 (http://www.treebase.org/treebase-web/home.html).

Bayesian inference (BI) was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The best substitution model for the alignment was estimated by both the

Table 1. Specimens included in DNA analysis. Sequences produced in this study marked in bold. For acronyms of biological provinces see e.g. Knudsen and Vesterholt 2008: Funga Nordica: 32–35. * = GenBank Accession Numbers

Species	Voucher	Herb	Locality	ITS*	rpb2*
C. bovarius (type)	11-188	H	U.S.A. Alaska, Fairbanks	**KC905156**	**KC905160**
C. bovarius	11-255	H	U.S.A. Alaska, Fairbanks	**KC905158**	**KC905162**
C. bovarius	11-298	H	Canada, Alberta, Hinton	**KC905157**	**KC905161**
C. bovarius	11-373	H	Canada, Alberta	**KC905159**	**KC905163**
C. bovinaster (type)	04-669	H	Finland, PeP, Ylitornio	JX407264	JX407340
C. bovinatus	09-1520b	H	Finland, ES, Kerimäki	JX407267	JX407341
C. bovinus	10-006	H	Norway, Oppl, Lunner	JX407282	JX407343
C. oulankaënsis	09-535	H	Norway, NTr, Steinkjer	JX407295	JX407345
C. anisatus	04-550	H	Finland, PeP, Runteli	DQ120754	JX407346
C. neofurvolaesus	04-001	H	Finland, U, Helsinki	DQ139997	JX407367
C. sordidemaculatus	04-003	H	Finland, U, Kirkkonummi	DQ139991	JX407368

Akaike information criterion and the Bayesian information criterion with jModelTest version 0.1.1 (Posada 2008). A GTR model, including a gamma shape parameter, was chosen for both DNA regions. Two independent runs with four chains in each were performed for 1 000 000 generations sampling every 100th generation. All trees sampled before stationarity were discarded with a 25% safety margin (burn-in of 2 500 trees [250 000 generations]). Sampled trees from both runs were combined in a 50% majority rule consensus phylogram and posterior probabilities (PP) were calculated. The analysis was run with computer clusters of the CSC, IT Center for Science, Espoo, Finland.

Morphological descriptions are based on material collected by the authors including specimens in all stages of development. Color notations in the description follow Munsell (2009) soil color charts. Microscopic characteristics were observed from dried material mounted in Melzer's reagent (MLZ). Measurements were made in MLZ with an ocular micrometer using 100× oil-immersion lens. Basidiospores were measured from the veil or top of the stipe, 20 spores from one basidiocarp. The length and width were measured for each spore, and their length/width ratios (Q value) were calculated. The lamellar trama and basidia also were examined, and the pileipellis structure was studied from scalp sections taken from the pileus center.

Results

The 50% majority rule phylogram resulting from the BI analysis is shown in Fig. 1. *Cortinarius bovarius* is supported as a new taxon (PP 1.00). It clusters together with *C. bovinus* (PP 0.90) but differs from it by at least 18 substitutions and indel positions in the ITS regions and 3 substitutions in the *rpb2* region. The four ITS sequences of *C. bovarius* have altogether 1 base and 2 length intragenomic polymorphisms. No sequences of this species exist in public databases.

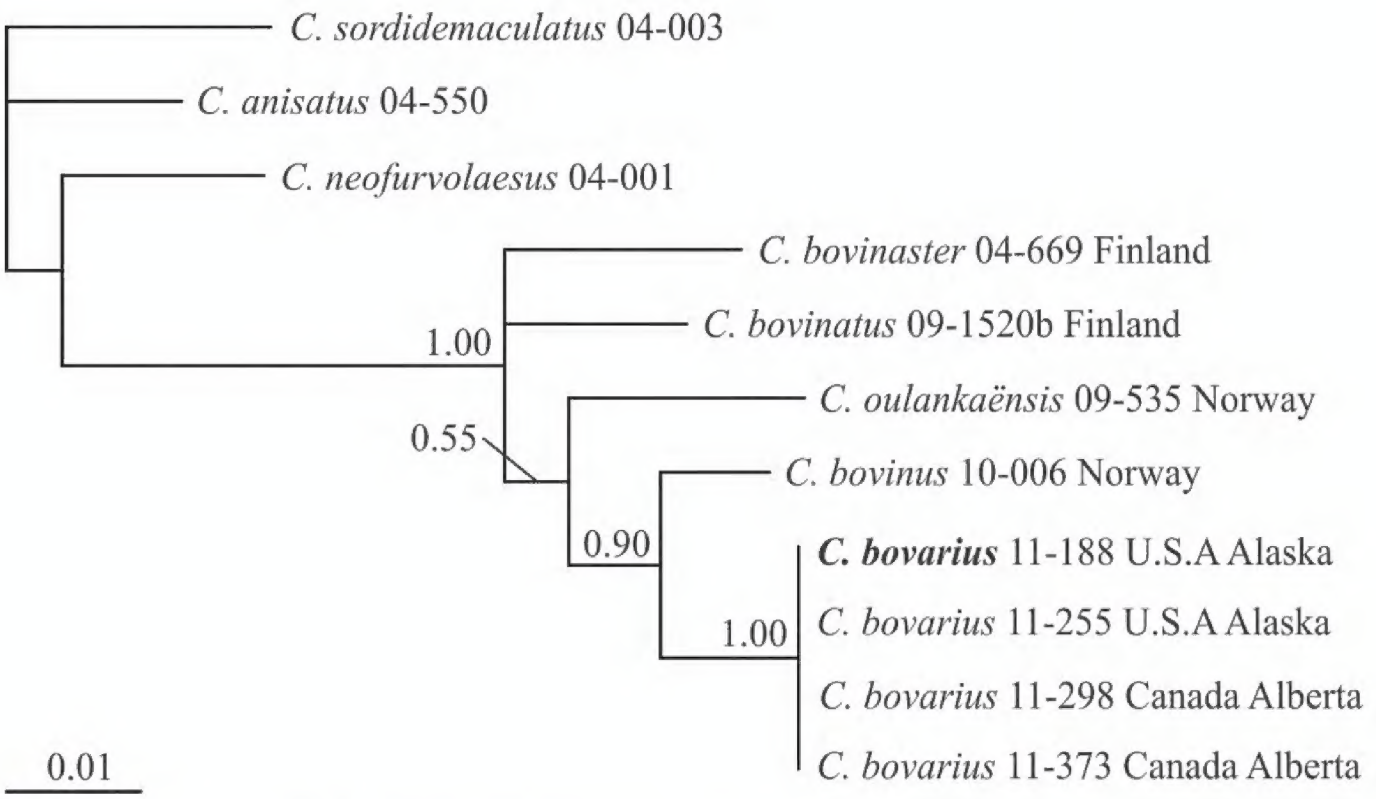

Figure 1. The Bayesian 50% majority-rule consensus tree inferred from combined ITS and *rpb2* regions. PP > 0.50 are indicated above branches.

Taxonomy

Cortinarius bovarius Liimat. & Niskanen, sp. nov.

MycoBank MB 804030
http://species-id.net/wiki/Cortinarius_bovarius
Figures 2 and 3

Diagnosis. Basidiomata medium-sized to large, pileus reddish brown, smell in lamellae indistinct or slightly raphanoid. Universal veil at first white, becoming pale brown. Basidiospores 8.5–10 × 5.5–6(–6.5) µm, amygdaloid to weakly ellipsoid. In coniferous forests with *Picea*, on rich, calcareous ground. Belongs to sect. *Bovini*.

Description. Pileus 3.5–7 cm diam., hemispherical at first, then low convex to almost plane, sometimes with a low, broad umbo, weakly fibrillose when young, later more apparent fibrillose only on the margin, somewhat waxy-glossy when moist; when young light reddish brown (5YR 6/4) to yellowish red (5YR 5/6-4/6) to reddish brown (2.5YR 5/4–4/4, 5YR 5/4–4/4), later dark red (2.5YR 3/6) to dark reddish brown (5YR 3/4–4/4, 2.5YR 3/3–3/4) and often with black spots; hygrophanous, soon drying from the center like *Kuehneromyces mutabilis* to lighter and more reddish brown, in dry condition reddish yellow (5YR 6/6, 7.5YR 7/6–6/6). Lamellae medium spaced to almost distant, adnexed to emarginate, fairly broad to broad, light reddish brown (5YR 6/4), light brown (7.5YR 6/3–6/4) to yellowish red (5YR 4/6), later dark reddish brown (2.5YR 3/4, 5YR 3/4–4/4), edge paler or concolorous. Stipe 5–11 cm long, 0.8–1.7 cm wide at apex, 1–3.5 cm wide at base, clavate to almost bulbose, rarely cylindrical, grayish white (silky) fibrillose, soon light reddish brown (5YR 6/3–6/4) to reddish brown (5YR 5/4) when older. Universal veil at first white, becoming pale

Figure 2. Photo of *Cortinarius bovarius* 11-188 (H). Photograph by K. Liimatainen.

brown, forming a girdle and thin sock-like sheath or rarely incomplete girdles on stipe surface, almost completely lost with age. Basal mycelium white. Context marbled hygrophanous, in pileus and upper part of the stipe light reddish brown (5YR 6/3–6/4) to reddish brown (5YR 4/4, 5/3), darkening towards the base of the stipe, in base reddish brown (5YR 5/3) when young, dark reddish brown (2.5YR 3/4 to 5YR 3/3–3/4) when old. Odor indistinct or slightly raphanoid. Exsiccatae: pileus brown (7.5YR 4/2–4/3) to dark brown (7.5YR 3/2–3/3), sometimes with a black center; stipe very pale brown (10YR 8/2) to light gray (10YR 7/2), in older basidiomes often darker, from grayish brown (10YR 5/2) to dark brown (10YR 4/2).

Basidiospores 8.5–10 × 5.5–6(–6.5) µm, Q = 1.45–1.65, av. = 8.9–9.5 × 5.7–6.1 µm, Qav. = 1.49–1.62 (80 spores, 4 specimens, Fig. 3), amygdaloid to weakly ellipsoid, moderately verrucose, somewhat more strongly so at the apex, moderately dextrinoid. Lamellar trama hyphae smooth to very finely scabrous, sometimes with sepia colored spots. Basidia 4-spored, 30–40 × 7.5–9.5 µm, almost concolorous with the background to olivaceous brownish. Pileipellis duplex, epicutis thin, hyphae 3–9 µm wide, unevenly pale brown, pigment in granules or in walls of hyphae, hypoderm distinct, elements 30–55 × 15–25(–30) µm, hyaline and smooth. Clamp connections present.

Ecology and distribution. In mesic coniferous forests with *Picea*, on rich, calcareous soil. Known from U.S.A, Alaska and Canada, Alberta. Fruiting from late August to September.

Etymology. *bovarius* for its affinity to *C. bovinus*.

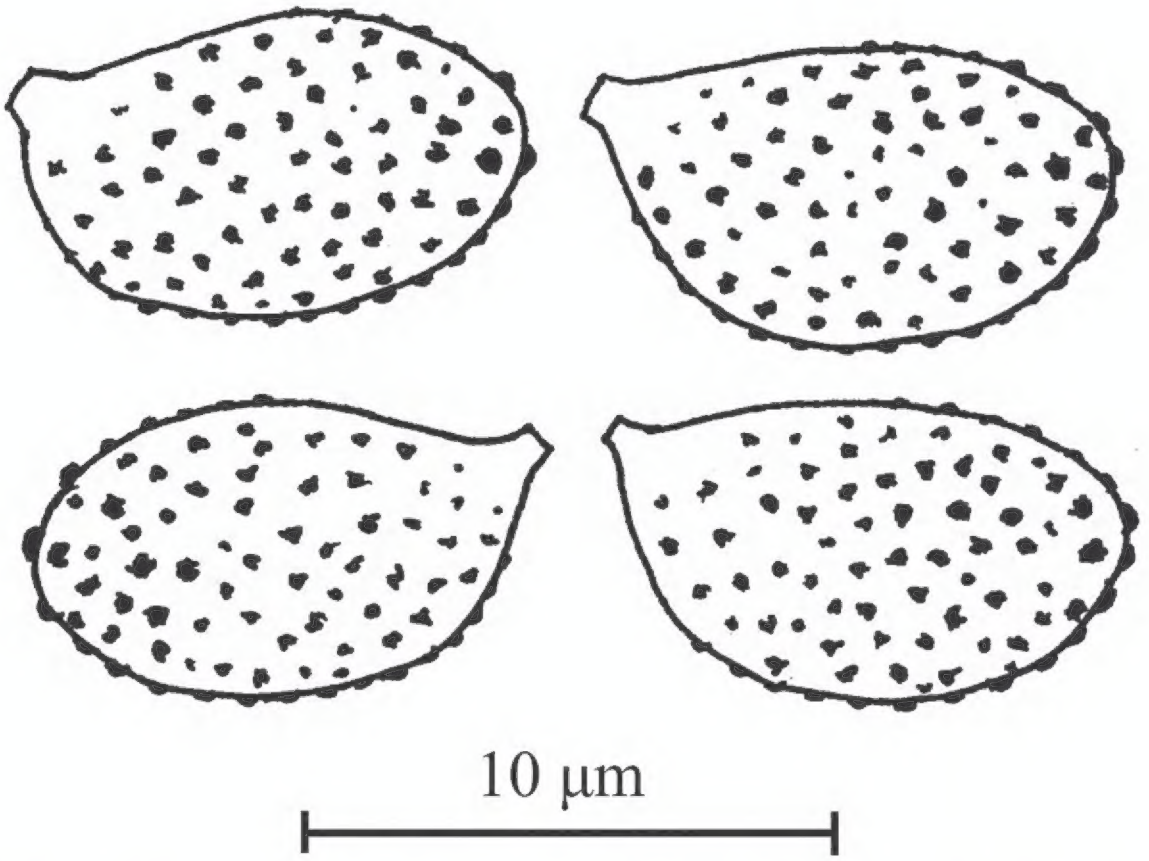

Figure 3. Spores of *Cortinarius bovarius* 11-188 (H) in Melzer's reagent. Drawing by T. Niskanen.

Type. U.S.A. Alaska: Fairbanks, University campus NW, trails starting from the end of Yukon road, mesic, mossy, partly needle/leaf covered *Picea* dominated forest with some *Populus, Betula, Alnus* and *Salix*, on rich ground, 64°51'33"N, 147°49'29"W, 22 Aug 2011, Niskanen & Liimatainen 11-188 (H, holotype; NY, isotype). GenBank no. KC905156 (ITS), KC905160 (*rpb2*).

Other specimens examined. Canada, Alberta, Hinton, S of center, Road to Percotte Creek, old mossy *Picea* dominated forest with some *Populus*, on calcareous ground, 53°21'53"N, 117°33'29"W, 30 Aug 2011, Liimatainen & Niskanen 11-298 (H). Alberta, Hinton, N of Athabasca river, *Populus* dominated forest with some *Picea*, 53°22'48"N, 117°51'35"W, 1040 m a.s.l., 5 Sept 2011, leg. L. Gagnon, Niskanen 11-373 (H). U.S.A. Alaska, Fairbanks, Wedgewood Resort trails, mesic *Picea* dominated forest with some *Betula* and *Populus*, on calcareous ground, 64°51'41"N, 147°42'46"W, 25 Aug 2011, Liimatainen & Niskanen 11-255 (H).

Discussion. *Cortinarius bovarius* is a typical member of section *Bovini*, a brown species with at first a white universal veil that later becomes brownish, indistinct or slightly raphanoid smell, and occurrence on calcareous ground. It differs from its European sister species, *C. bovinus*, by on average narrower, less dextrinoid and less verrucose spores (those of *C. bovinus* on average 6.1–6.4 µm wide, fairly strongly to strongly verrucose at the apex, and fairly strongly dextrinoid). The other known species of section *Bovini* s. str. from western North America, *C. oulankaënsis*, has a more grayish brown pileus, more distant lamellae, and relatively narrower spores (Qav. = 1.61–1.65). *Cortinarius bovarius* is a well-defined species based on morphology and molecular data, and therefore, is here describe as new to science.

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